REMARKS

I. Status of the Claims

Previous claims 58 and 61-109 are pending in the present application, claims 59, 60 and 110-112 having been cancelled without prejudice or disclaimer. Applicants reserve the right to file one or more applications directed to the cancelled subject matter.

Claim 58 has been amended to recite that the exon is defined at the 3' end by the splice donor site. Support for this amendment can be found in Applicants' specification *inter alia* on page 38 (see constructs (2)-(5)). Furthermore, Applicants submit that this limitation is implicit in that exon sequence inherently contains nucleotide sequence that is present in the processed message, which includes the sequence between the transcription start site and the splice donor site. Thus, by definition, the splice donor sequence would define the 3' end of an exon. Claim 58 has also been amended to point out that the selectable marker does not contain an operably-linked polyadenylation signal. Applicants believe that this was also implicit in the claim in that a polyadenylation signal would be understood to be a signal that allows polyadenylation. The claims now explicitly recite this limitation. Claim 58 has also been amended to incorporate the limitation in claim 59 that the first and second promoters are in the same orientation in the vector.

Claim 65 has been similarly amended to recite that the splice donor site defines the 3' end of the exon and also recites that neither the first nor second promoter contains an operably-linked polyadenylation signal.

Claim 67 has been amended similarly to recite that the second selectable marker sequence does not contain an operably-linked polyadenylation signal. The claim has also been amended to recite that the promoters are in the same orientation on the vector. Support for this amendment can be found in Applicants' specification *inter alia* in Figure 10 and the figure legend for Figure 10 found on page 19.

Claim 70 has been amended to recite that the positive and negative selectable marker and splice donor are oriented in the vector such that when the vector construct integrates "and the vector encoded splice donor is spliced to a splice acceptor" in the endogenous gene then the marker expression is affected. Support for this amendment can be found in Applicants' specification *inter alia* on page 79, lines 12-30.

The above claims and claim 73 have also been amended to recite that the vector "encodes" the marker sequence rather than stating that the vector contains a marker. Support for this amendment is implicit in the vector containing an expressible marker sequence.

Claims 79-94, 98-103 and 106 have been amended to recite that the host cells are *in vitro*. Support for this amendment can be found in Applicants' specification *inter alia* on page 7, line 25.

Claims 98, 100 and 101 have been amended to delete the term "genome-containing" with respect to cells in that it is inherent that if the vector integrates into the genome of the cell, as recited in the claims, then the cell inherently contains a genome.

Claim 99 has been amended to incorporate the first three steps of claim 98, on which claim 99 previously depended.

Clam 103 has been amended to incorporate the first four steps of claim 102, upon which previous claim 103 depended.

Claim 70 has been divided into two embodiments. Claim 70 is now limited to the embodiment wherein when splicing occurs the negative selectable marker is not expressed. New claim 118 is based on claim 70 and recites that when splicing occurs the selectable marker is expressed in inactive form.

Clam 97 has also been divided to recite that when splicing occurs the selectable marker sequence is not expressed (current claim 97) or when splicing occurs the selectable marker is expressed in inactive form (new claim 117).

Claim 100 has been amended to explicitly recite that a vector exon-tagged cDNA is a cDNA containing vector sequence and sequence from an endogenous gene and to more explicitly state that the endogenous gene sequence is used to recover exon I of the endogenous gene. Applicants believe that the new recitation merely states more clearly what was recited in previous steps (f) and (g). Accordingly, no new matter has been added with any of these amendments.

II. Response to Restriction Requirement

Applicants confirm the election of Group I, claims 58-109 and 113-116, pursuant to a telephone conversation held with Examiner Nguyen and election of the following species: dihydrofolate reductase for claim 76; Epstein Barr virus ori P for claim 77; BAC for claim 108; neomycin for claim 113 and thymidine kinase for claim 114.

III. The Rejections

A. The Rejection Under 35 U.S.C. § 112, First Paragraph

On page 5 of the Office Action, claims 58-109 and 113-116 are rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the claims are not enabled. Applicants traverse the rejection.

On page 8, the Examiner presents the grounds for rejecting independent claim 58 and claims 59-64, that depend on claim 58. First, the Examiner asserts that claim 58 is enabled only where the unpaired splice donor is located 3' from the exon. Accordingly, Applicants have amended the claim to recite that the exon is defined at its 3' end by an unpaired splice donor site.

On page 8 of the Office Action, the Examiner further discusses claim 58. The Examiner asserts that "[o]n the basis of the instant specification, it is unclear to Examiner what is the relevance of the second promoter operatively linked to a selectable marker encoding DNA sequence lacking a polyadenylation signal...". The Examiner questions how the selectable marker would be used and expressed. Applicants answer that the selectable marker will be effectively expressed if it is operably-linked to a polyadenylation sequence in an endogenous gene. Thus, a promoter operably-linked to a selectable marker lacking a functional polyadenylation signal provides a way to detect cells in which an integration event traps a polyadenylation sequence at the 3' end of a gene.

The Examiner is directed to the schematic labeled "Claim 58" in Appendix A and to the Figure Legend for Appendix A. The schematic shows the transcripts that are produced from the claimed vector when it integrates upstream of a single exon gene (A and C) and multi-exon gene (B and D). The schematic also shows the first promoter upstream of the second promoter (A and B) and downstream of the second promoter (C and D). Note that in every case, two independent transcripts are produced by the vectors. In A-D, the selectable marker sequence is transcribed in a polyadenylated transcript. Expression of the selectable marker, accordingly, shows that the marker is operably-linked to a polyadenylation signal on an endogenous gene.

For disclosure of polyadenylation trap activation vectors, the Examiner is directed, for example, to Applicants' specification, page 76, the section headed "Poly(A) Trap Activation Vectors". This section continues to the end of page 77, in relevant part. This text discloses the relevance of having a promoter and selectable marker lacking an operably-linked polyadenylation signal.

On page 8 of the Office Action, the Examiner asserts that "...it is unclear and uncertain ...that the selectable marker would be expressed and it would display its intrinsic selectable property, especially one has to take into considerations of the folding and the stability of such a large fusion mRNA message...".Applicants respectfully submit that it is reasonably predictable that the selectable marker will function in a large fusion mRNA.

First, the processed transcripts produced by the claimed vector are not significantly larger than other cellular mRNAs. Second, fusion mRNAs are routinely translated in eukaryotic cells, including mammalian cells. For example, in the art of recombinant gene expression, it is common to construct a vector that provides a chimeric transcript that expresses a marker allowing for selection in the host mammalian cell and that also expresses a desired protein. Moreover, eukaryotic messages typically span thousands of bases, where the coding sequences may be on exons thousands of bases apart. Nevertheless, the transcripts are translated. Furthermore, in multi-exon genes the

promoter may be tens of thousands of base pairs upstream from the coding sequence. In such cases, the coding sequence is translated in eukaryotic cells.

Applicants point out that the Examiner must provide evidence showing that it is *reasonably predictable* that the fusion transcript will prevent marker function. The Patent Office must meet this burden to establish a *prima facie* case of non-enablement. Applicants submit that it is not reasonably predictable that transcript size or hybrid form would prevent marker expression because large transcripts, including large fusion transcripts, normally allow translation.

On page 5 of the Office Action, the Examiner also rejects claim 58 on the grounds that it can be used only when the first and second promoter are oriented in the same direction. Applicants point out that this is not the case. There are instances of integration where the selectable marker, in opposite orientation, lacking an operably-linked polyadenylation signal, can be used to screen for cells that have activated transcription of a gene that is transcribed in a direction opposite from that of another gene that is activated by the first promoter/exon/splice donor. Nevertheless, to expedite prosecution, Applicants have cancelled claim 59 and incorporated into claim 58 the limitation wherein the first and second promoters are in the same orientation. They reserve the right to file one or more applications directed to the cancelled subject matter.

On page 9 of the Office Action, the Examiner explains the rejection of independent claim 65 and dependent claim 66. The Examiner bases the rejection on the

assumption that there must be an exon or selectable marker sequence operably linked to the first or second promoter. He asserts that "the present specification does not provide any teaching regarding the use of any vector having simply promoters without operably linked to any exon and an unpaired splice donor site". Applicants have amended the claim to explicitly recite that the splice donor site defines the 3' end of an exon. Applicants point out, however, that the person of ordinary skill in the art would have appreciated that any sequence between the transcriptional start site and splice donor inherently constitutes an exon, as defined, for example, in Applicants' specification on page 48.

The Examiner is directed to the schematic labeled "Claim 65". The schematic shows the transcripts that are produced from the claimed vector when it integrates upstream of a single exon gene (A and C) and multi-exon gene (B and D). A and B show the first promoter upstream of the second promoter and B and D show the first promoter downstream of the second promoter. Two independent polyadenylated transcripts are produced by each vector. Furthermore, transcription from a promoter that does not contain a splice donor still allows activation of an endogenous gene. Transcription from the promoter proceeds through the endogenous gene to the polyadenylation site of the endogenous gene. No splicing is required. The person of ordinary skill in the art would have been familiar with the function of the claimed components and would have, therefore, known how to use the vector, as claimed, to activate an endogenous gene.

Applicants further point out that the claim is generic. Therefore, it need not recite elements that are not necessary for generic function, such as a selectable marker. Applicants point out that detecting activation does not require a selectable marker. Gene activation can be detected in various other ways. The specification discusses this in detail. See, for example, page 49 starting at the first full paragraph and continuing through the first full paragraph of page 50.

The Examiner is directed to Applicants' specification on page 83, the section headed "Vectors For Isolating Exon I From Activated Endogenous Genes". Note that on line 26 it is indicated that one transcriptional regulatory sequence is linked to a splice donor site and the other is not. On page 84 it is indicated that two transcripts are formed. One of them is derived from the vector exon spliced to an internal exon of an endogenous gene. A second transcript is derived from transcription from the transcriptional regulatory sequence without the splice donor which transcribes into exon I (or a single exon). It is also indicated (first full paragraph page 84) that a selectable marker is optional on the vector.

On page 9 of the Office Action, the Examiner discusses independent claim 67 and dependent claims 68, 69 and 97. First, the Examiner asserts that the claims allow the use of each component on separate vectors. However, claim 67 clearly states that both components are on the vector. The claim is directed to (a) *and* (b). Therefore, both of the components are required on the vector.

On page 9 of the Office Action, the Examiner specifically addresses claim 97 (dependent on claim 67). The Examiner states "...the specification fails to teach how the positioning of the unpaired splice donor site upstream of the first selectable marker encoding DNA sequence would result in the expression of the selectable marker in an active form...". Applicants agree with the Examiner in that claim 97 recites that when the unpaired splice donor is positioned upstream of the selectable marker, the marker would actually not be expressed. When the splice donor is positioned suitably within the selectable marker, it might be expressed, but not in an active form. Applicants have amended the claim to divide out these two embodiments and relate the position of the splice donor to the form of expression. Thus, claim 97 is directed to one embodiment and new claim 117 is directed to the other.

On page 5 of the Office Action, claim 67 is further rejected on the grounds that the person of ordinary skill in the art could not use the vector unless the first and second promoters are oriented in the same direction. Applicants have discussed this issue above with respect to claim 58. For the same reasons, they submit that the person of ordinary skill in the art would know how to use the vector where the two components are in opposite orientations. In this case, activation of genes that are transcribed on either side of the integrated vector and in opposite orientations could be achieved. Nevertheless, in the interest of expediting prosecution in the present case, Applicants have amended the claim to recite this limitation and reserve the right to file one or more applications directed to the cancelled subject matter.

On page 10 of the Office Action, the Examiner addresses independent claim 70 and dependent claims 71, and 113-116. The rationale for the rejection is that "...the claims encompass any and all possible structural orientations for the combination of a first promoter, a second promoter and an unpaired splice donor site in the vector to attain the functional limitation recited in claim 70", and presumably that it would require undue experimentation to determine which orientations would produce the functional limitation. Applicants agree that there are structural requirements for meeting the functional limitation. But Applicants submit that the person of ordinary skill in the art would have known how the recited components function and therefore would have known how to arrange them to meet the claimed functional limitation.

On page 10 of the Office Action, the Examiner cites claim 115 as an example. He poses the question "...how would a negative selectable marker is either not expressed or is expressed in an active form (the limitation requires the positioning of an unpaired splice donor site 5' upstream of or within the negative selectable marker encoding DNA sequence, respectively) and at the same time the positive selectable marker is expressed in an active form in such a structural orientation?" With regard to claim 115, it appears that (1) the Examiner does understand how the components should be oriented in order to achieve the claimed functional limitation. This shows that the person of ordinary skill in the art would in fact appreciate the arrangements that would achieve the functional result. The Examiner understands that the splice donor is related to the negative selectable

marker such that when splicing occurs the negative selectable marker is no longer functional.

This observation aside, Applicants address the Examiner's specific question. The answer is that the positive selectable marker can be expressed in active form because it is independently transcribed. The Examiner is directed to the schematic in Appendix A labeled "Claim 70". See Construct B. The positive selectable marker is operably-linked to an independent promoter. Therefore, two transcripts are produced. One is a transcript from the transcriptional regulatory sequence that is operably linked to the negative selectable marker. When integrated upstream or within a multi-exon gene, splicing can occur between the splice donor on the vector and the splice acceptor on the endogenous gene. When this occurs, the negative selectable marker will not be expressed in active form. However, transcription can also occur from the transcriptional regulatory sequence operably-linked to the positive selectable marker and this transcription is independent of transcription from the other promoter. Accordingly, the positive selectable marker can still be expressed in active form from the independent transcript. Applicants submit that the person of ordinary skill in the art would have known that the claimed vector could be used to produce the products in the diagram.

Applicants have amended claim 70 to explicitly recite that when there is a splicing event, negative marker expression is affected. Applicants believe that this was inherent in the claim, but to expedite prosecution, the claim has been amended.

For a detailed description of the use of vectors claimed in the present application, the Examiner is directed to the introduction on page 75 headed "Vectors For Non-Targeted Activation of Endogenous Genes" which explains some of the uses of these vectors and to the text from page 76, line 18, through the bottom of page 88.

On page 6 of the Office Action, claims directed to cells and libraries have been rejected on the grounds that the person of ordinary skill in the art would not have known how to make and or use the cells or libraries except *in vitro*. Applicants submit that cells can be made *in vitro* and introduced into an organism *in vivo*. This may or may not involve gene expression for therapeutic effect. Applicants discuss this in detail immediately below. However, the proceeding argument notwithstanding, Applicants have amended the claims to recite that the cells and libraries are *in vitro*. Applicants reserve the right to file claims directed to *in vivo* expression.

In vivo uses were discussed in a Declaration by Dr. John J. Harrington, an inventor in the above-captioned application, submitted in Applicants' co-pending Application No. 09/479,122, with the Response dated September 13, 2001. Dr. Harrington discusses various ways in which cells introduced into an animal, and which produce protein in the animal, were used in the art. The opinions and conclusions in the Declaration were based on evidence in the form of scientific references available in the art at the time that Applicants' earliest priority application was filed. These references show that it was useful to introduce a cell into an animal to produce a desired protein

from that cell. Accordingly, based on discussion and evidence in the Declaration, methods for expressing protein from cells introduced into an animal had a use that was well-known to the person of ordinary skill in the art. A copy of the Declaration is attached hereto as Appendix B. The Examiner is also directed to the Applicants' discussion in the accompanying Response, attached hereto as Appendix C.

Since Applicants have shown credible, substantial, and well-established utility for using cells to produce a gene product *in vivo*, Applicants believe they have shown how to use the invention *in vivo*.

Applicants finally point out that the case law has established that a claim may embrace some inoperative embodiments without violating either § 101 or § 112-1. *Atlas Powder Company v. E. I. DuPont de Nemours and Co.*, 750 F2d 1569; 224 USPQ 409 (Federal Circuit 1984). According to this case, it is not the function of claims to specifically exclude possible inoperative embodiments. Where, however, a patent claim specifically calls for a result that does not occur, the claim may fail to meet the utility requirement. *Raytheon Co. v. Roper Corporation,* 724 F2d 951; 220 USPQ 592 (Federal Circuit 1983). In the present case, the Applicants' claim does not specifically call for a result which does not occur because it is not directed to cell therapy. Therefore, even if the claim is construed as encompassing an inoperative embodiment, under the cited case law, the claim meets the requirements of § 112-1.

On page 14 of the Office Action, claims 94 and 95 have been rejected on the grounds that the only way to "identify" a gene is by direct sequencing. Applicants respectfully disagree. A gene can be identified by function, by specific hybridization, and by immunological or biochemical means, for example.

On page 14, claims 98 and 99 are rejected on the grounds that cells could not be made, the genomes of which comprising the integrated vector, expressing both first and second selectable markers (as claimed in step (c)). Applicants direct the Examiner to Appendix A, the schematic labeled "Claim 97". When there is integration of the vector upstream from a single exon gene, a splicing event does not occur and, therefore, transcripts will be produced from both promoters and both selectable markers will be expressed. The limitations in claim 97 are merely *contingencies*. Thus, claim 97 recites that *when* splicing occurs, the selectable marker is expressed in inactive form or not expressed at all. In claim 98, directed to a method for isolating single exon genes, splicing does not occur when the vector integrates in or upstream of a single exon gene. Therefore, both markers are expressed.

In view of the above discussion and amendments, Applicants submit that all grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

B. Rejection Under 35 U.S.C. § 112, Second Paragraph

On page 15 of the Office Action claims 58-109 and 113-116 have been rejected under 35 U.S.C.§ 112, second paragraph, on the grounds that they are indefinite. Applicants respectfully traverse the rejection.

On page 15, claims 58, 67, and 70 and their dependent claims have been rejected on the grounds that the phrase "selectable marker" is indefinite because a marker refers to a protein. Applicants point out that the phrase is routinely issued. For example, see Appendix D, U.S. 5,733,761, claim 21. Nevertheless, Applicants have amended the claims to recite that the selectable marker is a coding sequence. Claims 73 and 76 have been similarly amended to recite that the DNA encodes an amplifiable marker.

On page 16, claims 58 and 62-64 have been rejected as indefinite on the grounds that it is unclear whether component (a) and component (b) are on the same vector. The claims clearly state that the vector comprises (a) *and* (b). This clearly points out that the vector contains both components.

On page 16, claims 65 and 66 have been rejected on the grounds that the phrase "said first promoter, but not said second promoter, is operably linked to an unpaired splice donor site" is unclear. The Examiner asserts: "Normally, a promoter is operably linked to an exon not to unpaired splice donor site. Is there an exon operatively linked to the first promoter or not?" Applicants submit that the question of exon sequences in

addition to the splice donor sequence is not relevant to the question of clarity. The metes and bounds are clear: if the person of ordinary skill in the art uses a vector where the first promoter but not the second promoter is operably-linked to an unpaired splice donor site, they would know that they were infringing the claim.

On page 16, claims 67-69 have been rejected on the grounds that the relationship between component (a) and component (b) is unclear. The Examiner asks whether they are structurally-linked in the same vector. Applicants assert, as discussed above with respect to claim 58, that the claim does recite that the components are on the same vector by reciting (a) *and* (b). The preamble states a "vector comprising [(a) and (b)]". This means that (a) and (b) are on the same vector.

On page 16 of the Office Action, claim 70 and its dependent claims have been rejected on the grounds that the phrase "said splice donor site...in inactive form..." is unclear. The Examiner states "Although the functional limitation is disclosed, it is unclear about the structural orientation of the splice donor site with respect to the first promoter and to the second promoter in order to attain the desired functional limitation". Applicants point out, however, that in order to achieve the functional limitation, constraints are imposed upon the claimed components. That is, the splice donor would have to be found in a specific position and it would be clear to the person of ordinary skill in the art where the splice donor must be located. The functional limitation in the claim has been amended to recite that the vector-encoded splice donor is spliced to a splice

acceptor in the endogenous gene and, as a consequence of the splicing, the negative selectable marker is not functional. To meet these limitations, the person of ordinary skill in the art would realize that the splice donor would need to be located either upstream of the negative selectable marker or in the negative selectable marker. Because the functional limitation imposes boundaries on the position of the splice donor that would have been clear to the person of ordinary skill in the art, Applicants respectfully submit that the specific position need not be recited.

On page 17, the Examiner raises the same issue with claim 71. Claim 71 contains an additional component: a third promoter linked to a second splice donor site. Applicants point out, however, that the position of this third component is not limited as long as the functional limitation is met. Applicants submit that the person of ordinary skill in the art would have understood that this claimed component could be found at any site on the vector as long as the functional limitation is met. That is, when splicing occurs from the vector-encoded splice donor to the endogenous splice acceptor, the negative selectable marker is not functional and the positive selectable marker is functional.

On page 17 of the Office Action, claims 98 and 99 (that depend on claim 97) are rejected as indefinite for reciting "in which said first and second selectable markers are expressed in their active forms" in step (c). The stated rationale is that when the vector of claim 97 is integrated into the genome, the first selectable marker is expressed in inactive

form or not expressed at all. Thus, the Examiner questions how both the first and second markers can be expressed in their active forms and especially when the splice donor is positioned within the first selectable marker "thereby disrupting the marker". The Examiner requests clarification.

First, Applicants point out that claim 97 does not *require* splicing . It recites a *contingency*. That is, *if* splicing occurs when the vector integrates, the marker is not functional or is inactivated. If splicing does not occur, both markers are capable of functional expression. Note that claim 97 recites that "*when* said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site...then said first selectable marker is expressed in inactive form or is not expressed at all". By reciting this condition, the person of ordinary skill in the art would understand the metes and bounds of the claim, i.e., that if there is no splicing, there is no inactivation.

The Examiner is directed to the schematic labeled "Claim 97". Constructs A and B show the transcripts that are produced when the vector integrates upstream of a dual exon gene (i.e., where splicing occurs). In this schematic, the splice donor is upstream of the selectable marker so when splicing occurs the marker sequence is processed out of the primary transcript. Constructs C and D show the transcripts that are produced when integration is upstream of a single exon gene. In this case, splicing does not occur so both markers can be expressed.

Note that claims 98 and 99 are directed to a method for activating a single exon gene. Thus, the person of ordinary skill in the art, being familiar with how the components on the claimed vector would function, would understand that when both markers are expressed in active form, this indicates that a single exon gene is activated. Accordingly, Applicants submit that the metes and bounds of the claim would have been clear to the person of ordinary skill in the art.

On page 17 of the Office Action, the Examiner states that in claim 99 the relationship between steps (d)-(f) and the isolation of cells in which a single exon gene has been activated is unclear. The Examiner states that the method for isolating cells as recited in claim 98 is already completed in step (c). The Examiner states that if Applicants intend to claim a method for isolating a single exon gene, it should be claimed independently. Accordingly, Applicants have amended claim 99 to incorporate limitations (a)-(c) in claim 98, while also amending the preamble to recite that the claim is directed to a method for isolating a single exon gene.

On page 18 of the Office Action, claim 100 has been rejected as indefinite on the grounds that "it is unclear which steps are involved in the recovery of the activated endogenous gene using said vector exon-tagged cDNA molecules". The Examiner requests clarification. Applicants point out that exon I is recovered by means of the sequence in the cDNA that corresponds to the activated gene. "Recovery", therefore, is necessarily generic and would have been understood by the person of ordinary skill in the

art to apply to any methods in which a nucleic acid sequence can be used to recover another nucleic acid sequence. In the present case, for example, it would be understood that the endogenous gene sequence that was obtained in the cDNA could be used to screen various cDNA libraries, to walk the chromosome in genomic DNA, or to analyze bioinformatically-obtained genomic sequences for upstream exonic sequences in the gene at issue. Applicants have amended the claim to more clearly recite that a sequence in the cDNA that corresponds to the activated gene is used to recover exon I sequence.

On page 18 of the Office Action, claim 102 has been rejected on the grounds that the phrase "otherwise combining with" renders the claim indefinite. The Examiner asserts that the metes and bounds of the claim are unclear because it is not clear which means, other than inserting the isolated genomic DNA into one of the recited vectors, is possible. Applicants point out that in step (c) a vector-genomic DNA complex is transfected into a host cell. The vector-genomic DNA complex can be ligated or be composed of independent fragments. The complex can become ligated in the cell. Accordingly, step (b) would have been understood by the person of ordinary skill in the art to encompass the case in which the vector and isolated genomic DNA is either pre-ligated or not, prior to transfection.

On page 18 of the Office Action, claim 103 is rejected as indefinite on the grounds that a method for producing a gene product (recited in the preamble of claim 102) is already completed and the steps in claim 103 are not related to a method for

producing a gene product. Accordingly, Applicants have incorporated steps (a)-(d) into claim 103 and amended the preamble to recite that the method is directed to a method for isolating a gene.

On page 18 of the Office Action, the Examiner rejects claim 116 as indefinite on the grounds that there is insufficient antecedent basis for "The cell of claim 115". Accordingly, the claim has been amended to correct this typographical error.

In view of the above discussion and amendments, Applicants submit that all grounds for rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

If the Examiner believes that a telephonic interview would expedite prosecution of this case, he is invited to contact Applicants' attorney, Anne Brown, at 216-426-3586 or Joseph Contrera at 703-683-6197.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Amended Claims with Marking to Show Changes Made"**.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 CFR § 1.136(a), and any fees required

therefor are hereby authorized to be charged to Deposit Account No. **50-0622**, referencing attorney Docket No. **0221-0003G**.

Respectfully submitted,

Anne Brown

Anne Brown
Registration No. 36,463

Shanks & Herbert
TransPotomac Plaza
1033 N. Fairfax Street #306
Alexandria, VA 22314
Phone: (703) 683-3600
Fax: (703) 683-9875

VERSION WITH MARKINGS SHOWING CHANGES MADE

In the Claims:

58. (Once amended) A vector comprising:

(a) a first promoter operably linked to an exon ~~and~~ defined at its 3' end by an unpaired splice donor site, and

(b) a second promoter operably linked to a sequence encoding a selectable marker ~~lacking a~~ that lacks an operably-linked polyadenylation signal~~.~~; wherein said first and second promoters are present in said vector in the same orientation.

65. (Once amended) A vector comprising a first promoter and a second promoter, said first and second promoters being oriented in the same direction, wherein:

(a) said first promoter, but not said second promoter, is operably linked to an exon defined at its 3' end by an unpaired splice donor site; and

(b) said vector comprises no operably-linked polyadenylation signals downstream of either said first promoter or said second promoter.

67. (Once amended) A vector comprising:

(a) a first promoter operably linked to a sequence encoding a first selectable marker ~~containing~~ and an unpaired splice donor site; and

(b) a second promoter operably linked to a sequence encoding a second selectable marker, wherein neither said first selectable marker sequence nor said second selectable marker sequence contains ~~a~~ an operably-linked polyadenylation signal~~.~~;

wherein said first and second promoters are present in said vector in the same orientation.

68. (Once amended) The vector of claim 67, wherein said first and second selectable ~~markers~~ marker sequences are positive selectable ~~markers~~ marker sequences.

69. (Once amended) The vector of claim 67, wherein said first selectable marker sequence is located upstream of said second selectable marker sequence.

70. (Once amended) A vector construct comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said positive and negative selectable ~~markers~~ marker sequences and said splice donor site are oriented in said vector construct in an orientation such that, when

said vector construct is integrated into the genome of a eukaryotic host cell ~~in such a way that~~ and the vector-encoded splice donor is spliced to a splice acceptor in an endogenous gene in said genome ~~is transcriptionally activated~~, then said positive selectable marker sequence is expressed in active form and said negative selectable marker sequence is ~~either~~ not expressed ~~or is expressed in inactive form~~.

73. (Once amended) The vector of any one of claims 58, 65, 67, 70, or 71, said vector further comprising sequences encoding one or more amplifiable markers.

79. (Once amended) A host cell *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

80. (Once amended) A host cell *in vitro* comprising the vector of claim 72.

81. (Once amended) A host cell *in vitro* comprising the vector of claim 73.

82. (Once amended) A host cell *in vitro* comprising the vector of claim 74.

83. (Once amended) A host cell *in vitro* comprising the vector of claim 75.

84. (Once amended) A host cell *in vitro* comprising the vector of claim 78.

87. (Once amended) A library of cells *in vitro* comprising the vector of any one of claims 58, 65, 67, 70, or 71.

88. (Once amended) A library of cells *in vitro* comprising the vector of claim 72.

89. (Once amended) A library of cells *in vitro* comprising the vector of claim 73.

90. (Once amended) A library of cells *in vitro* comprising the vector of claim 74.

91. (Once amended) A library of cells *in vitro* comprising the vector of claim 75.

92. (Once amended) A library of cells *in vitro* comprising the vector of claim 78.

93. (Once amended) A method for activation of an endogenous gene in a cell *in vitro* comprising:

(a) transfecting a ~~genome containing~~ cell *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71; and

(b) culturing said cell under conditions suitable for non-homologous integration of said vector into the genome of said cell, wherein said

integration results in the activation of an endogenous gene in the genome of said cell.

94. (Once amended) A method for ~~identifying a~~ obtaining cDNA from an endogenous gene comprising:

(a) transfecting a plurality of ~~genome containing~~ cells *in vitro* with the vector of any one of claims 58, 65, 67, 70, or 71;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of the ~~host~~ cell;

(c) selecting for cells in which said vector has integrated into the genomes of said cells;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA; and

(f) ~~identifying a gene in said cDNA by~~ isolating one or more cDNA molecules containing one or more nucleotide sequences from said vector.

95. (Once amended) The method of claim 94, wherein said ~~identification in (f)~~ isolation is accomplished by hybridizing said cDNA to said vector.

96. (Once amended) The method of claim 94, wherein said ~~identification in (f)~~ isolation is accomplished by sequencing said cDNA and comparing the nucleotide sequence of said cDNA to the nucleotide sequence of said vector.

97. (Once amended) The vector of claim 67, wherein said unpaired splice donor site is positioned upstream of ~~, or within,~~ said first selectable marker sequence such that, when said vector is integrated into the genome of a eukaryotic host cell resulting in splicing from said unpaired splice donor site to a genome-encoded splice acceptor site, then said first selectable marker sequence is ~~expressed in inactive form or is~~ not expressed ~~at all~~.

98. (Once amended) A method for isolating cells *in vitro* in which a single exon gene has been activated, comprising:

(a) transfecting a plurality of ~~genome-containing~~ eukaryotic cells *in vitro* with the vector of claim 97;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genomes of said cells; and

(c) selecting for cells in which said first and second selectable ~~markers~~ marker sequences are expressed in their active forms.

99. (Once amended) ~~The method of claim 98, further~~ A method for isolating a single exon gene cDNA comprising:

(a) transfecting a plurality of eukaryotic cells *in vitro* with the vector of claim 97;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genomes of said cells;

(c) selecting for cells in which said first and second selectable marker sequences are expressed in their active forms;

(d) isolating RNA from the selected cells;

(e) producing cDNA from said isolated RNA; and

(f) isolating a single exon gene from said cDNA.

100. (Once amended) A method for isolating exon I of a gene comprising:

(a) transfecting one or more ~~genome containing~~ eukaryotic cells *in vitro* with the vector of any one of claims 58, ~~59,~~ 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has transcriptionally activated an endogenous gene containing one or more exons;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA;

(f) recovering a cDNA ~~molecules~~ molecule containing ~~a first exon from said vector spliced to a second exon~~ vector sequence and sequence from said endogenous gene ~~, thereby obtaining one or more vector exon-tagged cDNA molecules~~; and

(g) using said endogenous gene sequence to recover exon I of said endogenous gene ~~using said vector exon-tagged cDNA molecules to recover the activated endogenous gene containing exon I~~.

101. (Once amended) A method for expressing a transcript containing exon I of a gene, said method comprising:

(a) transfecting one or more ~~genome containing~~ eukaryotic cells *in vitro* with the vector of any one of claims 58, ~~59,~~ 61, 65, or 67;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells; and

(c) culturing said cells under conditions suitable for expression of a transcript containing exon I from an endogenous gene.

102. (Once amended) A method for producing a gene product encoded by an endogenous cellular genomic gene, comprising:

(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) inserting into or otherwise combining with said isolated genomic DNA, the vector of any one of claims 58, ~~59~~, 61, 65, or 67, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable eukaryotic host cell *in vitro*; and

(d) culturing said host cell under conditions suitable to result in transcription of one or more genes encoded by said vector contained in said vector-genomic DNA complex.

103. (Once amended) ~~The method of claim 102, further~~ A method for isolating a gene sequence comprising:

(a) isolating genomic DNA, containing at least one gene, from a eukaryotic cell;

(b) inserting into or otherwise combining with said isolated genomic DNA, the vector of any one of claims 58, 61, 65, or 67, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable eukaryotic host cell *in vitro*;

(d) culturing said host cell under conditions suitable to result in transcription of one or more genes encoded by said vector contained in said vector-genomic DNA complex;

(e) isolating RNA produced by said transcription from said host cell;

(f) producing one or more cDNA molecules from said isolated RNA; and

(g) recovering one or more cDNA molecules containing vector sequences at the 5' ends of said cDNA molecules, thereby isolating said gene sequence.

106. (Once amended) A method for producing a protein comprising:

(a) isolating genomic DNA from one or more cells;

(b) inserting into or otherwise combining with said isolated genomic DNA, the vector of any one of claims 58, ~~59,~~ 61, 65, or 67, thereby producing a vector-genomic DNA complex;

(c) transfecting said vector-genomic DNA complex into a suitable host cell *in vitro*; and

(d) culturing said cell under conditions suitable to result in protein expression from said genomic DNA contained in said vector-genomic DNA complex.

113. (Once amended) The vector construct of claim 70, wherein said positive selectable marker sequence is selected from the group consisting of a neomycin gene, a hypoxanthine ~~phosphribosyl~~ phosphoribosyl transferase gene, a puromycin gene, a dihydrooratase gene, a glutamine synthetase gene, a histidine D gene, a carbamyl

phosphate synthase gene, a dihydrofolate reductase gene, a multidrug resistance I gene, an aspartate transcarbamylase gene, a xanthine-guanine phosphoribosyl transferase gene, and an adenosine deaminase gene.

114. (Once amended) The vector construct of claim 70, wherein said negative selectable marker sequence is selected from the group consisting of a hypoxanthine ~~phosphribosyl~~ phosphoribosyl transferase gene, a thymidine kinase gene, and a diphtheria toxin gene.

115. (Once amended) The vector of claim 70, wherein said negative selectable marker sequence is located upstream of said positive selectable marker.

116. (Once amended) The ~~cell~~ vector of claim 115, wherein said vector further comprises one or more selectable ~~markers~~ marker sequences.

FIGURE LEGEND FOR APPENDIX A

↱:	promoter
EX:	exon
S/D:	splice donor
S/A:	splice acceptor
SEL:	selectable marker
pA:	poly(A) site
NEG:	negative selectable marker
POS:	positive selectable marker

The horizontal lines under the constructs are unprocessed transcripts that are transcribed from each promoter. The darkened region corresponds to the part of the transcript that is spliced out in the processed transcript. The open boxes represent single exon genes. The hatched boxes represent exon I and exon II of a dual exon gene.

CLAIM 58

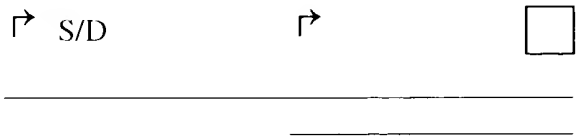
A. EX S/D SEL pA

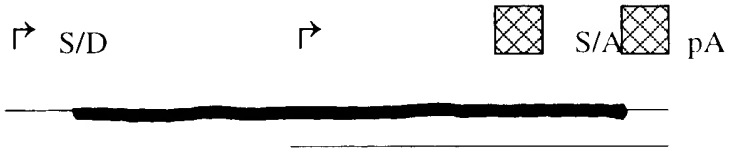
B. EX S/D SEL S/A pA

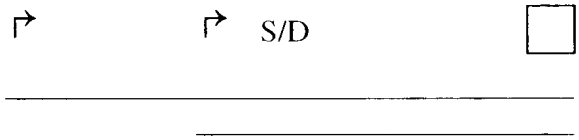
C. SEL EX S/D pA

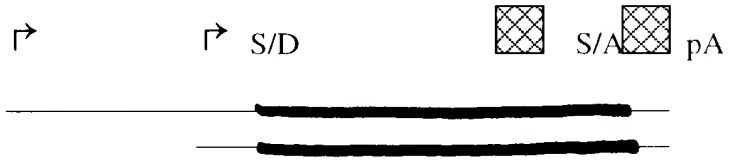
D. SEL EX S/D S/A pA

CLAIM 65

A. 

B. 

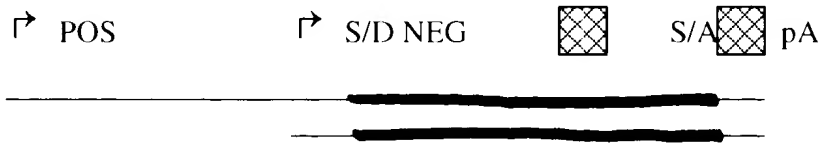
C. 

D. 

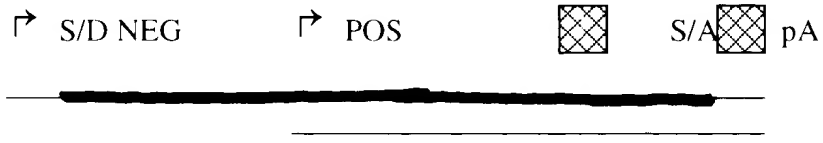
c:\\public\anne\0221-0003\Claim 65 Schematic

CLAIM 70

A.



B.



c:\\public\anne\0221-0003G\Claim 70 Schematic

CLAIM 97

A. S/D SEL SEL S/A pA

B. SEL S/D SEL S/A pA

C. S/D SEL SEL pA

D. SEL S/D SEL pA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPENDIX B

In re Application of:
Harrington, *et al.*

Serial No.: 09/479,122

Filed: January 7, 2000

For: **COMPOSITIONS AND METHODS FOR NON-TARGETED ACTIVATION OF ENDOGENOUS GENES**

Group Art Unit: 1632

Examiner: Brunovskis, P.

Attorney Docket No.: 0221-0003C

Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

The undersigned, John J. Harrington, declares and states:

1. I am an inventor of the above-captioned patent application, U.S. Application No. 09/479,122, filed January 7, 2000, entitled "Compositions and Methods for Non-Targeted Activation of Endogenous Genes." I am the subject of the attached Curriculum Vitae and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am an expert in the field of non-homologous recombination, eukaryotic gene expression and gene cloning and am qualified to speak on the skill and knowledge of the person of ordinary skill in these fields.

2. I have read and understand the subject matter of the above-captioned application. I have read and understand the Office Action dated March 15, 2001, rejecting claims 75, 81 and 83–88 under 35 U.S.C. § 112, first paragraph, on the grounds that it would have required an undue burden of experimentation to practice the claimed invention. It is my opinion, based on the scientific evidence and reasoning below, that the methods that are the subject of the rejected claims could have been made and used by the person of ordinary skill in the art, as of the filing date of September 26, 1997 (the earliest effective filing date), by routine and ordinary experimentation, using the Applicants' specification and general knowledge in the art as a guide.

3. It is my opinion, based on the scientific evidence and reasoning set forth below, that the methods that are the subject of the rejected claims provided a practical, real-world use. I assert this because the methods provide a way to use cells to produce protein in an animal and using cells to produce protein in an animal had a practical, real-world use as of the Applicants' earliest effective filing date.

4. It is my further opinion that the person of ordinary skill in the art reading Applicants' specification would have immediately appreciated that producing protein from Applicants' cells in an animal was useful, as of the Applicants' earliest effective filing date. I assert this because cell-based protein production in an animal was known by the art to be useful and the claimed methods were disclosed as a way to provide cell-based protein production in an animal.

5. As a rationale for the rejection, the Examiner has stated that the specification discloses only one real-world use for the claimed method: cell therapy. I do not agree with this statement because the specification discloses cell-based protein production in an animal and there was real-world use for cell-based protein production in various contexts besides cell therapy. Some of these will be discussed further below in this Declaration. In addition, the specification also discloses the isolation and purification of protein produced in an animal by the cells of the invention. This disclosure clearly demonstrates a utility distinct from cell therapy, since cell therapy does not involve subsequent purification of the protein following expression *in vivo*. Isolation and purification of proteins produced in an animal had real-world use. Thus, the specification does disclose a process with real-world use in addition to use for cell therapy.

The Examiner has also asserted that he is unaware of any well-established utility for the claimed method except cell therapy. I, therefore, point out that there was well-established utility for the claimed method for uses in addition to cell therapy.

I understand a well-established utility to be a real-world use that would have been immediately apparent to the person of ordinary skill in the art reading the Applicants' specification. It is my opinion that both non-therapeutic, cell-based protein production in an animal and isolation of proteins produced from cells placed in an animal would have been recognized immediately as having practical use by the person of ordinary skill in the art.

6. My opinions and conclusions in this Declaration are supported by evidence in the form of scientific references that I will discuss in the paragraphs that follow. These references show that there were non-therapeutic uses for introducing a cell into an animal to produce a desired protein from the cell. The Applicants' disclosure directs the artisan to introduce Applicants' cells into an animal to produce protein from the cell. Having access to the references, the person of ordinary skill in the art would have readily appreciated that the claimed methods also had non-therapeutic utility.

7. The following references, available as of the earliest effective filing date, show some non-therapeutic practical uses for producing protein from cells introduced into an animal.

8. Brodeur et al., Kints et al., and Stewart et al. demonstrate the utility of expressing a protein from a cell introduced into an animal. The utility is not based on cell therapy. Specifically, each of these authors describes a method for introducing hybridomas into mice or rats to produce large quantities of antibodies. The antibodies are produced from endogenous antibody genes in the hybridomas. The purpose of this work was to optimize conditions for producing antibodies so that the antibodies could be purified. It is stated in these references that there are advantages to producing antibodies *in vivo*. The references thus demonstrates a utility for protein production *in vivo* that does not involve cell-based therapy.

The Applicants' specification discloses and claims methods for expressing desired protein from endogenous genes in eukaryotic host cells and subsequently introducing the cells

into an animal to produce protein *in vivo*. Applicants' specification, in fact, also discloses using hybridomas to express endogenous genes using the methods of the invention. See U.S. Application No. 08/941,223, page 30, line 22 and U.S. Application No. 09/276,820, page 53, line 29. The specification also discloses expression of antibodies using Applicants' methods. See U.S. Application No. 08/941,223, page 22, line 25 and U.S. Application No. 09/276,820, page 43, line 16. The specification also discloses isolating and purifying proteins expressed *in vivo*.

Based on these similarities, it is my opinion that it would have been readily apparent to someone skilled in the art that Applicants' cell lines and hybridomas, expressing endogenous antibodies, could be used to produce antibodies *in vivo*. It is, therefore, my opinion that there was practical, well-established utility for using the claimed method.

9. U.S. Patent No. 5,733,761 describes uses for production of proteins *in vivo*. The uses are not based on cell therapy. Desired endogenous proteins are activated by homologous recombination. In column 3, starting at line 17, it is indicated that the cells introduced into the animal are useful for eliciting antibody production or for immunizing humans or animals against pathogenic agents. The antigens can be used to produce antibodies that are then used for therapeutic or diagnostic purposes. This U.S. patent is a continuation of U.S. Application No. 07/985,586. Accordingly, it contains the same specification. WO 94/12650 is a PCT application claiming priority to U.S. Application No. 07/985,586. The relevant text in the U.S. application, discussed above, is found in the PCT application on page 5. The PCT application was published

in June 1994. Accordingly, this information was available to the person of ordinary skill in the art well before the earliest effective filing date of Applicants' claims.

The patent specification discloses non-therapeutic uses in addition to those discussed directly above. In column 14, starting at line 1, the disclosure discusses implanting cells for agricultural use, for example, meat and dairy production. It is my opinion that this disclosure would have been recognized by the person of ordinary skill in the art to include such uses as the delivery of hormones to an animal through the implanted cells. Line 6 then goes on to discuss the implanted cells as also being useful for eliciting antibody production for immunizing humans and animals against pathogenic agents or for producing antibodies useful for therapeutic and diagnostic purposes. This discussion can be found in the corresponding PCT application on pages 27 and 28.

U.S. Patent No. 5,641,670 has a corresponding PCT application, WO 95/31560, published November 23, 1995. In the U.S. patent, column 3, starting at line 27, the specification discusses the use of homologously recombinant cells to immunize animals or produce antibodies in immunized animals. The patent specification also discusses agricultural uses, citing the production of bovine growth hormone for dairy production. See column 4, lines 37–39. Starting at line 47, the specification also discusses *in vivo* immunization and use of the cells to produce antibodies for diagnostic and therapeutic purposes. In column 18, line 40, the disclosure discusses implanting the cells for agricultural uses (i.e., meat and dairy production).

Accordingly, both U.S. Patent Nos. 5,641,670 and 5,733,761, and their corresponding PCT applications, demonstrate utility for production of protein *in vivo* that is not based on cell therapy. It is my opinion that it would have been readily apparent to the person of ordinary skill in the art that the protein-producing cells disclosed in Applicants' specification had the same non-therapeutic utility. It is my opinion, therefore, that the claimed method had practical, well-established utility.

10. Shaw et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell-based therapy. The reference describes the introduction of cells expressing IL-4 and IL-10 into a mouse. The purpose of this work was to produce IL-4 and IL-10 protein *in vivo*. Another purpose of this work was to test the biological activity of the proteins in a disease model. Finally, the authors state that *in vivo* production of protein from introduced cells allows them to produce the proteins at a specific site in the animal, as opposed to systemic delivery of an injected protein.

The Applicants' application also describes introducing cells expressing a protein of interest into an animal to produce protein. The Applicants' specification describes activation and expression of a variety of proteins, including IL-4 and IL-10. See U.S. Application No. 08/941,223, page 22, line 30; page 23, line 10; and page 48, line 16 and U.S. Application No. 09/276,820, page 43, lines 21–23.

Based on these similarities, it is my opinion that using the claimed method to produce IL-4 and IL-10 *in vivo* would have been readily apparent. It would also have been apparent from this reference that producing protein in an animal by the present method is useful to test the biological activity of the protein *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

11. Chen et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the nerve growth factor gene into normal fibroblasts, and subsequently introducing the cells expressing NGF into the nucleus basalis magnocellularis (i.e. a region of the brain) of rats. Following implantation, the rats were tested using a Morris water maze to assess their spatial memory ability. The authors show that expression of NGF *in vivo* can reverse naturally occurring age-related memory loss. The authors state that production of protein in an animal "can be used both to explore basic biological questions concerning the structure and function of the brain or as a form of somatic gene therapy. A principal advantage of this approach is the local intraparenchymal delivery of factors to responsive cells, which allow one to examine the effects of the factors on specific populations of cells. Additionally, following the implantation of the transfected cells there is no need for any further invasive procedure, such as the chronic infusion of various factors into the cerebral ventricles by osmotic minipump." Thus, it was appreciated that *in vivo* expression of a protein is useful to define a biological process and potentially as a therapeutic. This art also appreciated the advantages of expressing a protein *in vivo*, as opposed to introducing the purified protein into the animal.

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce the protein. It also describes activation and expression of a variety of proteins, including nerve growth factors. See U.S. Application No. 08/941,223, page 23, line 6; page 25, line 24; and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. It further describes the use of primary cells and fibroblasts to express a protein of interest. For disclosure of primary cells, see U.S. Application No. 08/941,223, page 30, lines 2 and 13–15 and U.S. Application No. 09/276,820, page 53, lines 9 and 20–22. For fibroblasts, see U.S. Application No. 08/941,223, page 30, lines 7 and 21 and U.S. Application No. 09/276,820, page 54, line 15. It also describes use of any eukaryotic cell, including rat cells. For rat cells, see U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that using the claimed method to produce nerve growth factor *in vivo* would have been readily apparent. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to study biological processes including memory and cognition. It is my opinion, therefore, that the claimed invention had practical, well-established utility.

12. Garver et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the human alpha 1-antitrypsin (alpha 1AT) gene into normal mouse fibroblasts, and subsequently

introducing the cells expressing alpha 1AT into the peritoneal cavities of mice. The authors show that human alpha 1AT could be detected in the sera and epithelial surface of the lungs, and that upon recovery, the mouse fibroblasts continued to express alpha 1AT four weeks following introduction into the animal. The authors state that production of protein in an animal is useful as a model for gene therapy and as an "approach to study the *in vivo* effects of such hormones and growth factors."

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. It also describes activation and expression of a variety of proteins, including alpha 1AT. See U.S. Application No. 08/941,223, page 22, line 24; page 23, line 3; and page 48, line 19 and U.S. Application No. 09/276,820, page 43, line 26. It also describes the use of primary cells and fibroblasts to express a protein of interest. It describes use of any eukaryotic cell, including mouse cells. See U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the Applicant's method to produce alpha 1AT *in vivo*. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to "study the *in vivo* effects of such hormones and growth factors," or other genes. It is my opinion, therefore, that the claimed method had practical, well-established utility.

13. McNiece et al. demonstrates the utility of producing protein from a cell placed in an animal for other than cell therapy. This reference reports the introduction of a cell line, WEHI-3, into mice to produce large amounts of IL-3. IL-3 was expressed from the endogenous IL-3 gene in the cell line. Following introduction into the animal, IL-3 activity was detected in both the sera and ascites fluids of the mice. The IL-3 protein was subsequently purified. In the last paragraph, page 1074, the reference states "The WEHI-3 tumor-bearing mice may thus provide a model for the study of the effects *in vivo* of SF and IL-3 on bone marrow cells." The reference thus demonstrates a utility for protein production *in vivo* other than for cell-based therapy.

Applicants' specification discloses introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins, including cytokines, and specifically interleukins. For interleukins, see above. For cytokines, see U.S. Application No. 08/941,223, page 22, line 24 and U.S. Application No. 09/276,820, page 43, line 15. It also describes the use of a variety of cell lines similar to WEHI-3.

Based on these similarities, it is my opinion that a person of ordinary skill in the art would have recognized that Applicants' method could be used to produce IL-3 *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

14. Ishihara et al. demonstrates the utility of expressing a protein from a cell placed in an animal for other than cell therapy. The reference describes the *in vivo* production of a protease by introducing a tumor cell line, AH109A, into rats. The protease was produced from the endogenous protease gene in the tumor cell line. The purpose of the experiment was to assess the effect of protease expression on tumor cell invasiveness. The protease was also isolated from serum protein and purified 1150-fold to assess and characterize the protease produced *in vivo*.

The Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins. These include, but are not limited to, proteases, including TPA, urokinase, and protein C. For TPA, see U.S. Application No. 08/941,223, page 22, line 27 and page 48, line 14 and U.S. Application No. 09/276,820, page 43, line 19. For urokinase, see U.S. Application No. 08/941,223, page 23, line 6 and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. For protein C, see U.S. Application No. 08/941,223, page 23, line 4 and page 48, line 20 and U.S. Application No. 09/276,820, page 43, line 27. It also describes the use of a variety of tumor cell lines. These can include a hepatoma cell line, Hep G2, similar to the hepatoma AH109A tumor cell line. For Hep G2, see U.S. Application No. 08/941,223, page 30, line 20 and U.S. Application No. 09/276,820, page 53, line 27. The specification states that cell lines useful for activating endogenous genes can be derived from any tissue. Liver cells and hepatoma cells are specifically cited. For hepatoma cell lines, see U.S. Application No. 08/941,223, page 30, line 21 and U.S. Application No.

09/276,820, page 53, line 28. For liver cells, see U.S. Application No. 08/941,223, page 30, line 4 and U.S. Application No. 09/276,820, page 53, line 11.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the claimed method to produce proteases, such as TPA, urokinase and protein C, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

15. Bronson et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. The reference reports introducing the bcl-2 gene into mouse embryonic stem cells by homologous recombination. The embryonic stem cells were injected into blastocysts, and subsequently introduced into a pseudopregnant mouse using standard transgenic procedures. Bcl-2 was used as a test gene to establish the feasibility of this transgenic approach.

The Applicants' application describes introducing cells expressing a protein of interest into an animal to produce protein. The application describes activation and expression of a variety of proteins. The present invention describes the use of a variety of cell types including cells isolated from an embryo and stem cells. For embryo cells, see U.S. Application No. 08/941,223, page 30, line 6 and U.S. Application No. 09/276,820, page 53, line 13 and page 54, line 14. For stem cells, see U.S. Application No. 08/941,223, page 30, line 8 and U.S. Application No. 09/276,820, page 53, line 14 and page 54, line 16.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the present method to produce a desired protein, as a test gene for transgenics, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

16. The cited references cumulatively show various art-known uses of cell-based expression of a desired protein in an animal besides cell-based therapy. This evidence demonstrates real-world and well-established utility for cell-based protein expression in an animal that is not cell-based therapy. Since the referenced methods were useful and would have been recognized as useful, Applicants' claimed methods also would have been useful and would have been recognized as such.

Furthermore, because *in vivo* cell-based protein production was not confined (in the literature) to one or two specific proteins or classes of protein, I believe that the person of ordinary skill in the art would have realized that *in vivo* cell-based expression could be useful for any number of proteins. This being the case, they would have also realized that Applicants' methods would also be useful for a desired protein and not just a limited class.

SUMMARY

In summary, the literature shows that cell-based protein production in an animal was useful in a variety of contexts unrelated to cell-based therapy. Therefore, non-therapeutic methods using Applicants' cells to produce protein in an animal also were useful.

The relevant literature on non-therapeutic uses for cells expressing protein in an animal was available to the person of ordinary skill in the art at the earliest effective filing date. Therefore, the person of ordinary skill in the art would have known of these uses as of this date. Accordingly, as of this date, it would have been readily apparent that Applicants' cells could be used non-therapeutically. Thus, there was a well-established non-therapeutic utility for Applicants' methods.

John J. Harrington, Ph.D.

9/5/01
Date

APPENDIX C

Credible Utility for In Vivo Protein Production

"Credible utility" is based on "whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided." Applicants respectfully submit that the utility of the production of a desired protein in an animal would have been believable to the person of ordinary skill in the art at the time that the application was filed based on the evidence and discussion in the Declaration.

Well-Established Utility

"Well-established utility" is defined as "a specific, substantial and credible utility, which is well-known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art." Utility Guidelines Training Materials, page 7. Applicants submit that the using cells non-therapeutically to produce a desired protein in an animal had well-established utility at the time of Applicants' earliest filing.

The issue of well-established utility is discussed in the attached Declaration. Scientific evidence showing well-established utility is in the form of references from the scientific literature available to the person of ordinary skill in the art at the time that Applicants' earliest application was filed. The references show that non-therapeutic *in vivo* expression of proteins from implanted cells was a developed art at the time of filing. Accordingly, the person of ordinary skill in the art would have known of the non-

therapeutic utility of protein expression from cells introduced into an animal. Applicants' specification teaches protein expression from cells introduced into an animal. Because the non-therapeutic utility of protein expression from cells introduced into an animal was known to the person of ordinary skill, the non-therapeutic utility of Applicants' methods would have been readily apparent.

Thus, the Examiner is respectfully directed to the attached Declaration for the discussion and evidence supporting these assertions. Based on the evidence in the Declaration, Applicants believe that they have met their burden of showing that the non-therapeutic use of Applicants' claimed methods was a well-established utility.

Substantial Utility Disclosed

The Examiner has also asserted that the only real-world use *disclosed* in the specification was for cell therapy. Applicants respectfully disagree. The isolation and purification of proteins produced by cells introduced into an animal *in vivo* is disclosed in Applicants' specification. These have a real-world use. References discussed in the Declaration are evidence of this use.

However, Applicants point out that even if the isolation and purification of these proteins had not been disclosed, the claimed method still would have had a well-established utility. Please see the section headed "Well-Established Utility."

Assertions of Utility in U.S. Application No. 08/941,223

Applicants' specification discloses the utility of isolating the protein produced *in vivo*. For the Examiner's convenience, the relevant text is given below.

Page 7, lines 3–9

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desirable amounts of the gene product of the endogenous gene whose expression has been activated or increased. The gene product can then be isolated and purified.

Alternatively, the cell can be allowed to express the desired gene product *in vivo*.

Page 8, lines 10–17

- The invention also encompasses methods for using the cells described above to overexpress a gene that has been characterized (for example, sequenced), uncharacterized (for example, a gene whose function is known but which has not been cloned or sequenced), or a gene whose existence was, prior to over-expression, unknown. The cells can be used to provide desired amounts of a gene product *in vitro* or *in vivo*. The gene product can then be isolated and purified if desired. It can be purified by cell lysis or from the growth medium (as when the vector contains a secretion signal sequence).

Page 9, lines 4–9

- The invention accordingly is also directed to methods of using libraries of cells to overexpress endogenous genes. The

library is screened for the expression of the gene and cells are selected that express the desired gene product. The cell can then be used to purify the gene product for subsequent use. Expression in the cell can occur by culturing the cell *in vitro* or by allowing the cell to express the gene *in vivo*.

Page 13, lines 1–2

- The methods are also capable of producing over-expression of known and/or characterized genes for *in vitro* or *in vivo* protein production.

Page 16, lines 9–15

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desired amounts of the gene product of the endogenous gene that has been activated or whose expression has been increased. The gene product can then be isolated and purified to use, for example, in protein therapy or drug discovery.

Alternatively, the cell expressing the desired gene product can be allowed to express the gene product *in vivo*.

Pages 16–17, lines 25–30 and 1–2

- The cell over-expressing the gene is cultured such that amplification of the endogenous gene is obtained. The cell can then be cultured *in vitro* so as to produce desired amounts of the gene product of the amplified endogenous gene that has been activated or whose expression has been increased. The gene product can then be isolated and purified.

Alternatively, following amplification, the cell can be allowed to express the endogenous gene and produce desired amounts of the gene product *in vivo*.

Page 17, lines 11–17

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desirable amounts of the gene product of the endogenous gene whose expression has been activated or increased. The gene product can then be isolated and purified.

Alternatively, the cell can be allowed to express the desired gene product *in vivo*.

Page 29, lines 24–25

- Cells produced by this method can be used to produce protein *in vitro* (e.g., for use as a protein therapeutic) or *in vivo* (e.g., for use in cell therapy).

Page 35, lines 21–26

- The invention is also directed to methods of using libraries of cells to over-express an endogenous gene. The library is screened for the expression of the gene and cells are selected that express the desired gene product. The cell can then be used to purify the gene product for subsequent use. Expression of the cell can occur by culturing the cell *in vitro* or by allowing the cell to express the gene *in vivo*.

As is clear from the above text, isolation and purification of the protein produced in the animal are disclosed. The utility was substantial and also would have been readily

apparent to the person of ordinary skill in the art. See the references addressed in the Declaration that disclose isolating and purifying protein produced *in vivo*.

Accordingly, the specification asserts utilities other than cell therapy that are specific, substantial, credible, and well-established.